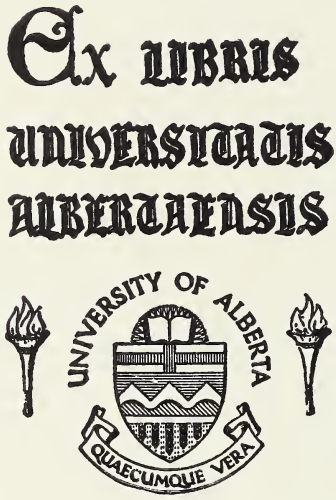

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THE EFFECT OF CERTAIN PSYCHOTHERAPEUTIC AGENTS ON ADENOSINE TRIPHOSPHATE IN SOME ORGANS OF THE RAT

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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by

JOHN DOUGLAS DUDAR, B.Sc.

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ABSTRACT

The effects of four psychotherapeutic agents upon the incorporation of radio-phosphorus into adenosine triphosphate, and the concentration of adenosine triphosphate have been studied, in the brain, liver, heart and adrenal glands of male rats.

The drugs used were: chlorpromazine, 25 mg/kg of body weight; reserpine, 2.5 mg/kg; chlordiazepoxide 50 mg/kg; and tranylcypromine, 10 mg/kg. The first three of these drugs are considered to be tranquillizing or depressant drugs, while tranylcypromine is classed as an anti-depressant.

Chlorpromazine administration did not significantly change the adenosine triphosphate (ATP) concentration in any of the tissues studied. It produced a reduction in the specific activities of liver, heart and adrenal glands and an increase in the specific activity of brain tissue. This compound significantly increased the plasma specific activity, suggesting that the transfer of the radio-phosphorus from the plasma to the intracellular fluid was impaired, perhaps due to an alteration in membrane permeability.

Reserpine, chlordiazepoxide, and tranylcypromine produced no significant changes in the brain, but lowered the concentration of ATP and the specific activity in the other tissues. This suggests that the synthesis of ATP has been reduced in these tissues.

The results obtained would indicate that the psycho-therapeutic drugs studied do not manifest their action by

interfering with brain phosphorylative metabolism, but that they do interfere with the production of ATP in other tissues, chlorpromazine excepted.

ACKNOWLEDGEMENTS

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INTRODUCTION

From his genesis, man has searched for substances that would alter his psyche; either to release him from reality or to equilibrate him with it. His quest has been mingled with magic and mystical implications which have influenced empirical observations and therapeutic aims. The situation remained relatively unchanged until recently, when the almost simultaneous introduction of two psychotherapeutic agents, reserpine and chlorpromazine, wrought changes in the treatment of mental disease and stimulated interest in brain metabolism.

Reserpine, isolated from the root of Rauwolfia serpentina, and chlorpromazine, produced from the manipulation of the phenothiazine molecule in the search for a better antihistamine, are prototypes for the tranquillizing or ataractic drugs. These and other psychotherapeutic agents provide only symptomatic medication for the facilitation and management of disease states, as they neither cure mental illness nor do they remove the causes of anxiety. To date, their clinical manifestations are relatively well-known, but knowledge regarding their biochemical and physiological mechanisms of action is still inadequate.

Many attempts have been made to ascertain the mode of action of the various psychoactive drugs. Research has centered on the sites of action in the brain, as well as the metabolic processes within the cells themselves. Of particular interest are the enzyme systems and related functions which are necessary for the production and transfer of energy through adenosine triphosphate (ATP).

The intention of this work is to investigate the effect

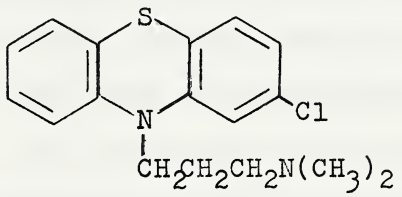
of several psychotherapeutic agents on the levels of ATP in various tissues, as well as the incorporation of radio-phosphorus into the ATP molecule. This should provide some further measure of the effects of these agents on ATP synthesis and/or utilization.

SURVEY OF THE LITERATURE

CHLORPROMAZINE

I PHARMACOLOGY

Chlorpromazine is the 2-chloro-10-(3-dimethylamino-propyl) derivative of phenothiazine, and can be represented structurally as follows:



It was found in 1953 (1) that chlorpromazine possessed potent anti-emetic and tranquillizing effects, as well as adrenolytic, hypotensive, hypothermic, antispasmodic and antihistaminic effects. It could also potentiate the action of several other pharmacological agents. Additional work by many other researchers has borne out these findings, and in doing so, has stimulated investigations into the intricacies of cerebral function.

Tangri and Bhargava (2) have stated that the locus for the hypotensive action of chlorpromazine seems to be the hypothalamus, while others (3) conclude that it is, in part, due to depression of the medullary vasomotor centre.

Kollias and Bullard (4) have shown that chlorpromazine abolishes mechanisms for temperature regulation of both heat and cold. A specific CNS site, possibly the hypothalamus, is thought to control these mechanisms. The hypothermia induced by chlorpromazine might be responsible for the inhibition of protein synthesis of the brain and other tissues observed in vivo by Shuster and Hannam (5). Bonaccorsi, Garattini, and Jori (6)

postulate that the hypothermia produced is responsible for hyperglycemia observed in rats, through an impaired utilization and an increased mobilization of glucose.

Chlorpromazine reduces conditioned responses and spontaneous psychomotor activity (7), as well as decreases muscle tone (8). This conclusion adds emphasis to the belief that chlorpromazine acts on the central areas of the nervous system. It appears that the study of psychological effects will be of little aid in the elucidation of the mode of action, until such time as the neurophysiological mechanisms involved in the process of learning are better understood.

Chlorpromazine has an effect on adenohypophyseal function, mediated through the central nervous system (9). It increases the secretion of ACTH (10,12), and inhibits the release of vasopressin (11). Chlorpromazine potentiates the adrenal-pituitary response to stress, producing adrenal insufficiency (12).

The unique pharmacological action of chlorpromazine would appear to involve selective inhibition of the subcortical centers of the CNS, including the reticular system of the brain, the thalamus, the hypothalamus, and the autonomic nervous system, while not significantly affecting the cortex (13), although alertness and spontaneity are reduced. It is believed that the drug acts principally on the higher neural centers in the general area of the diencephalon, selectively inhibiting the chemoreceptor trigger zone. These areas apparently control vomiting, heat regulation, wakefullness, vasomotor tone, muscle tone, and secretion from the anterior lobe of the pituitary (14). Many of the encephalographic and electrode studies carried out in this regard

support these suggestions (15,16).

A study of the localization of phenothiazine derivatives in fourteen areas of dog brain was undertaken by de Jarmillo and Guth (17). It found that those areas thought to be the loci of action of chlorpromazine did have the greatest concentration of the drug. However, it is difficult to correlate drug concentration and sites of action unless there is a very special receptor found solely at the site of action. Only then would it be reasonable to state that the area of highest concentration produces the greatest response.

The multiple pharmacological effects of chlorpromazine on mammalian tissues would tend to preclude a specific enzyme interaction to explain the overall mechanism. It is possible that a selectively enhanced cell membrane permeability is the common factor reconciling clinical, physiological, and pharmacological observations (18). Guth and Spirtes (11) support the hypothesis that the effect of the phenothiazines on the permeability of a great variety of membranes may serve to explain many of the actions of these drugs, and to unify many of the findings in this field.

Effects such as the antagonism of epinephrine (19,20, 12), histamine (21,12), serotonin (22,12), and acetylcholine (23, 12) may be due to an inhibition of the passage of these neurohumors to their sites of action. Substances such as histamine, serotonin, and acetylcholine some of whose effects are due to increases in permeability, would tend to have an antagonistic effect with a phenothiazine-induced decrease in permeability (11).

Forrest and Forrest (18) postulate that the mechanism of chlorpromazine is a selective action on membrane permeability

mediated by the positive ion radical which acts as an electron donor. This corresponds well with the findings of Szent-Györgyi (24), who found that chlorpromazine possesses extraordinary properties as an electron donor. He reasons that the action of chlorpromazine is related to its ability to transfer charges onto nerve cell molecules for which it has an affinity. Cotzias and Borg (25) found that the metastable free radical is itself chemically labile, spontaneously oxidizing and yet being readily reduced. It is presumably, then, able to alter cellular metabolic processes in sites where it is generated. However, it may not be that the radical itself is so potent, but rather its formation may change the valence of available cellular metal ions, which are themselves cofactors critically regulating important enzyme reactions.
The preparation and characterization of a solid, stable , free radical of chlorpromazine by Merkle, Discher, and Felmeister (26), may provide the means for further elucidation of this phenomenon.

II EFFECT ON ADENINE NUCLEOTIDES

From the gross observation that animals under the influence of chlorpromazine are indifferent to incoming stimuli, it is reasonable to hypothesize that CNS depressants operate, in part, by modifying the energy-producing cycles of mammalian brain metabolism. In order for such a hypothesis to be tenable, it must admit the action of psychotherapeutic drugs, in vivo, is associated with an alteration in either the concentration or turnover of ATP.

The numerous biological processes affected by chlorpromazine render explanations of the primary pharmacological actions exceedingly difficult. The metabolic factors affecting ATP can, however, be grouped into two categories: the first includes those factors which are believed to be primarily involved with oxidative phosphorylation and the synthesis of ATP; and the second, those factors which require the utilization of ATP. Inhibition of one or more reactions in the first group would be expected to result in an interference with the phosphorylation of the adenine nucleotides in brain tissue, and a consequent limitation or reduction of the supply of available energy for cerebral function. On the other hand, inhibition of those factors in the second category would lead to an impairment in the utilization of ATP, resulting in a possible accumulation of the compound. Thus, if chlorpromazine exerts its action on the first group, a reduction in the level of labile phosphate would be expected, and if utilization is inhibited, either directly or indirectly, unaltered or elevated levels would be expected.

Chlorpromazine has been shown to affect many of the

processes associated with energy production, particularly mitochondrial activity. These include inhibition of oxidative phosphorylation (27), dinitrophenol-activated and Mg^{++}-activated adenosine triphosphatase (ATPase) (27, 28), diphosphopyridine (DPN or nicotinamide adenine dinucleotide - NAD) cytochrome-c oxidase (29), and cytochrome oxidase (29, 30). It has also been found to inhibit mitochondrial swelling (11) but not electron transport-dependent swelling (31). Chlorpromazine is an inhibitor of oxidations coupled to phosphorylation (32) and has been found to complex with the flavins (33).

It is possible then, for chlorpromazine to react with the flavoprotein catalysing DPNH oxidation as suggested by Löw (27) and by Dawkins, Judah, and Rees (28). Since ATPase is also inhibited, then the participation of the phosphorylated flavoprotein in either the phosphorylation of ADP or the cleavage of the high energy phosphate bond by ATPase would be inhibited. This would lead to a reduction in the synthesis of ATP, as the electron transport system would not be functioning.

Grenell, Mendelson, and McElroy (34), examining the effects of chlorpromazine on brain ATP levels of the rat, found an initial increase in ATP levels followed by a return to control levels. Those areas thought to be the site of action of the drug had the highest concentration of ATP. However, in their subsequent work (35) using a different extraction technique, they found no changes in ATP levels between control and test animals. They concluded that chlorpromazine may in some way alter the distribution and binding of ATP, making it more difficult to extract. This alteration in binding might lead to an interference with ATP

utilization in specific metabolic systems, possibly those of protein synthesis, since the chief binding alteration appeared to be in the microsomal fraction of cells.

Weiner and Huls (36), utilizing a quick freezing technique to minimize post-mortem degradation of labile phosphate compounds, showed a difference in nucleotide levels between decapitated and whole animal freezing techniques. If the rats were sacrificed by decapitation and the head dropped into liquid oxygen, examination showed that pretreatment with chlorpromazine, in a wide range of doses, resulted in higher brain concentrations of ATP. However, when the whole animal was frozen, no demonstrable change in nucleotide levels took place. They concluded that enzymatic studies on brains of animals depressed with chlorpromazine revealed unaltered activities of creatine phosphokinase and enzymes catalysing dephosphorylation of adenine nucleotides. This suggests that chlorpromazine, in some way, depresses the utilization of ATP so that a given stimulus results in less utilization of labile phosphate. Moreover, the normal levels of labile phosphate compounds in chlorpromazine-depressed animals indicate that the biochemical effects of the drug, involving interference with oxidative enzyme systems and uncoupling of oxidative phosphorylation, are unrelated to the primary pharmacological action of the drug.

Albaum and Milch (37), using a similar freezing technique, studied the uptake of C^{14}-labelled acetate into brain ATP, and the influence on it of chlorpromazine and other drugs. They found that chlorpromazine markedly inhibits the incorporation of C^{14}-acetate into the adenine nucleotides of the rat brain. If such incorporation adequately reflects the rate of synthesis of ATP,

then energy available to support useful work in the cellular elements is sharply curtailed by chlorpromazine. In considering this, the possiblility that the observed results may be due to differences in the size of the acetate pool or to dilution of the ATP pools must be ruled out to validate the hypothesis. These researchers also felt that the rate of ATP breakdown and the rate of synthesis of ATP precursor are part of a homeostatic mechanism that tends to maintain a constant level of ATP.

Work done by Diamond (38), on the effects of chlorpromazine upon the phosphorus metabolism in the rat, showed that high doses of this drug decreased the incorporation of P^{32} into lipid and RNA phosphorus. Also, four hours after drug administration the incorporation of P^{32} into ATP phosphorus was increased in the adrenals and liver, decreased in the heart, and unchanged in the brain. After sixteen hours there was no change of incorporation into adrenals or liver, a decrease in the heart, and an increase in the brain of rats. It was concluded that the pharmacological actions of the drug are not due to its effects on phosphorylative metabolism.

A recent study by Kaul, Lewis, and Livingstone (39) was carried out on the influence of chlorpromazine on the levels of adenine nucleotides in the rat brain and hypothalamus *in vivo*. In general, they found that the levels of ATP in the drug-treated animals varied from control values in the same direction for both the hypothalamus and whole brain; there being a significant decrease in 3 hours and a significant increase in 6 hours after drug administration. They also found that the changes noted in brain and hypothalamic levels were not paralleled by changes in

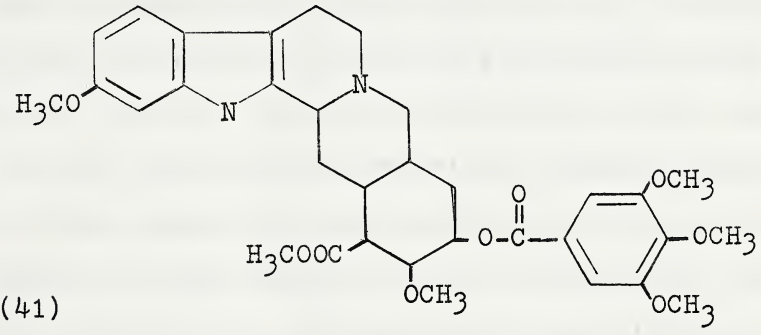
rectal temperature. Therefore, hypothermia has no marked effect on labile phosphate levels in the whole brain.

Kaul and his colleagues (39), in explaining their results, point out that chlorpromazine in low concentrations uncouples oxidative phosphorylation in rat brain mitochondria, and at higher concentrations inhibits ATPase activity. Thus, if the initial concentration of chlorpromazine is low, and only uncoupling can take place, the concentration of ATP will fall, provided that the rate of utilization is unchanged. As chlorpromazine concentration increases ATPase activity is inhibited, the rate of breakdown fails to equal the rate of synthesis, and so ATP levels rise. By this time, the animals are severely depressed such that the utilization of ATP is probably reduced as well.

RESERPINE

I PHARMACOLOGY

Reserpine, the best known alkaloid from Rauwolfia serpentina, was first isolated by Meuller and associates in 1952 (40). It may be represented structurally as follows:



(41)

The effects observed following reserpine administration are due to a complex of central and peripheral actions. The combination of hypotension, hypometabolism, and somnolence suggested the hypothalamus to be the site of action. More recent observations, on the ability of reserpine to cause the release of serotonin and norepinephrine from their binding sites, have introduced considerable changes in the original hypothesis. It is possible that the mechanism of action of reserpine is that of tranquillizing and sedation due mainly, if not entirely, to an action on the brain. The antihypertensive actions may be due to effects upon stores of catecholamines in the heart, arteries, and various other sympathetic peripheral sites.

The hypotensive effects of reserpine are in some measure linked directly or indirectly with an action upon peripheral stores of noradrenaline (43). Reserpine does have some direct

peripheral actions (42) which can be demonstrated upon isolated tissues and organs, but in the intact animal these appear to be of limited significance. Reserpine does have an effect on the hypothalamus, although reports are contradictory concerning the hypotensive action of reserpine related to the hypothalamus(15). Possibly a diminution of central sympathetic tone may be the primary cause of hypotensive action and this may be reinforced by the ability of reserpine to deplete peripheral stores of norepinephrine in blood vessels and adrenergic nerve endings.

Most of the major and important pharmacological actions of reserpine have generally been assumed to be due to actions upon the central nervous system, and are consequently thought to be closely linked to an influence upon brain levels of serotonin, dopamine, and noradrenaline. It is not certain that changes in brain levels of these compounds reflect the primary point of drug action, since there is also evidence for an action upon brain levels of adenine nucleotides, which themselves may play a role in the metabolism of the catechol amines and alkyl amines (44). Evidence that sedation is primarily linked to metabolism, storage, or release of brain catechol amines or indole amines is not yet conclusive (45).

Domino, in his review of the electropharmacology of reserpine (15), concludes that reserpine has a complex action on the hypothalamus showing both stimulant and depressant effects. The hypothalamus seems to be the logical site of action in view of the present knowledge of the relatively high content of catechol amines and serotonin in this area. In small doses, reserpine appears to have negligible effects on the cerebral cortex,

brainstem reticular formation, or spinal cord.

Reserpine has been shown to act on the pituitary-adrenal axis in a manner similar to the classical stress response, attributed to the release of ACTH (46). Westermann, Maickel, and Brodie (47) have demonstrated that the release of ACTH by reserpine is related to the blockade of monoamine storage in the brain, and that the hypersecretion of ACTH possibly results from the action of reserpine on neuronal pathways that monitor the anterior pituitary.

There is also evidence for a relationship between the metabolism of adenine nucleotides and the liberation of the catechol amines from the adrenal medulla. Carlsson, Hillarp, and Waldeck (48) have shown that amine granules of the adrenal medulla are able to take up and concentrate in vitro monoamines. The uptake is dependent on a Mg^{++}-ATP storage mechanism and blocked by low doses of reserpine. Other workers (49) have demonstrated that the release of catecholamines from the adrenal medulla granules is also dependent on a Mg^{++}-ATP complex.

Work by von Euler and Lishajko (50) showed that the release and uptake of catechol amines is dependent on ATP, and that reserpine can inhibit the ATP-dependent uptake. These findings corroborate the work of Kirpekar, Lewis, and Goodlad (51) who found that reserpine depletes the adrenal medulla of rats of both catecholamines and ATP. They reasoned that since both the catechol amines and ATP disappear in approximately the same proportions, the storage or release of catecholamines points to a specific role for ATP. There is, however, no such conclusive evidence for such a mechanism regarding cerebral catechol amines and ATP, and this remains an area of great conjecture.

II EFFECT ON ADENINE NUCLEOTIDES

The studies of Abood and Romanchek (52) have shown that reserpine acts as an uncoupling agent to oxidative phosphorylation in rat brain mitochondria. Kirpekar et al (51) found that reserpine lowered ATP levels of both rat liver and brain, suggesting inhibition of oxidative phosphorylation since reserpine had no effect on the ATPase of rat liver mitochondria. Bonasera, Mangrove, and Bonavita (53) have demonstrated the effect of reserpine in the mouse brain. Reserpine was found to modify the rate of penetration and exit of various precursors of DPN (NAD) through the blood-brain barrier and to interfere with DPN turnover in brain as well as in liver.

Albaum and Milch (37) in their study of the uptake of C^{14}-acetate into cerebral ATP found that reserpine elevated the specific activity of isolated ATP above the levels of the control animals. This suggested to them a higher rate of ATP synthesis rather than an inhibition of energy production. They felt that the psychotherapeutic effects of reserpine are achieved through an entirely different pathway than energy production, perhaps mediated by the catechol amines.

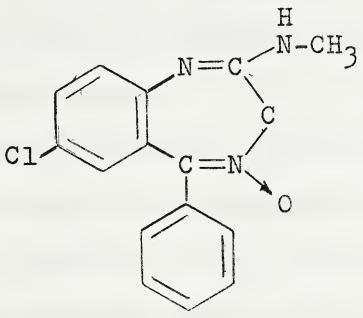
A recent study on the effects of reserpine on the levels of adenine nucleotides in the rat brain was conducted by Kaul and Lewis (44). Freezing the rat brain in situ, by immersing the rat in liquid nitrogen, they found that reserpine caused a significant decrease in the ATP/ADP ratio, as a result of an increase in ADP and a decrease in ATP levels. The ATP/ADP ratio can be regarded as an indication of the relationship between utilization and the production of energy within the cell, and can reflect either a

decreased synthesis or an increased utilization of ATP. Increased utilization of high energy phosphates is normally associated with emotional excitement caused by application of external stimuli. As this is not likely to occur in deeply sedated animals, a decrease in ATP synthesis is more probable. They state that the levels of ATP have not yet been correlated with levels of catechol amines, indolealkyl amines, or with acetylcholine; nor has there been established a relationship between the levels of the former to those of the latter group. It is possible that central nervous system stimulants and depressants act primarily on phosphate metabolism, and that observed changes in brain amines are secondary to the metabolic changes resulting from this action.

CHLORDIAZEPOXIDE

I PHARMACOLOGY

Chlordiazepoxide was the first therapeutic agent of the 1,4 benzodiazepine type to be discovered. It may be structurally represented as:



(54)

Chlordiazepoxide has been shown to possess sedative, muscle-relaxant, and anti-convulsant properties, but it exhibits weak hypnotic effects(55). It is thought that the anti-anxiety properties of the compound are due to a depression of the limbic system, while the muscle-relaxant properties are attributed to spinal reflex blocking (54). Since it has been suggested that the limbic system is associated with the hypothalamus, this may partially explain the hypothermia observed, as well as the anti-emetic effects (55). An advantageous property of chlordiazepoxide is its lack of autonomic blocking effects (56), as moderate doses have no effect on blood pressure, heart rate, or on blood pressure responses to carotid occlusion, serotonin, epinephrine , acetylcholine, or vagal stimulation.

Chlordiazepoxide was reported to have analgesic properties similar to aminopyrine, as demonstrated in an elevated pain threshold of a yeast-inflamed rat's foot (55). It was also reported to have the same potency as aminopyrine as an anti-edemic

and anti-pyretic agent.

Boris, Costello, Gower, and Welch (57) found that chlordiazepoxide exhibited no endocrine effects. They concluded that chlordiazepoxide does not possess gonadal-like hormonal activity, anti-gonadal hormone activity, or anti-thyrotropic activity, nor does it interfere with normal functioning of the pituitary-gonadal axis.

Monoamine oxidase activity of rat liver mitochondria was not inhibited by chlordiazepoxide (54), nor was DOPA decarboxylase, or the activity of acetylcholinesterase of rat brain. Other research (58), in which no changes in heart or brain bioamines were observed at dosage levels of 100 mg/kg, tends to substantiate this.

II EFFECT ON ADENINE NUCLEOTIDES

Sternbach, Randall, and Gustafson (54) report that chlordiazepoxide produced an increase in blood glucose levels after large doses were given to rats. They found that the elevation of glucose levels was not causally related to sedation. Rutishauser (59) also noted an elevation in blood glucose in rats, an intracellular increase in glucose concentration of brain, and a decrease in brain pyruvate levels. From this information it would appear that chlordiazepoxide interferes with glycolysis.

Kadenbach and Lührs (60) found that chlordiazepoxide promoted the swelling of rat liver mitochondria, but not rat brain mitochondria. This effect on rat liver mitochondria could be reversed by the addition of Mg^{++} and ATP. They also showed that chlordiazepoxide inhibited oxidation linked to DPN but not to succinate. The phosphorylation associated with DPN-linked substrates is not affected, but in the case of succinate there is a depression. Chlordiazepoxide depressed DPN-induced ATPase in both brain and liver mitochondria, suggesting that it inhibits the redox reactions between the flavoprotein and reduced DPN and not the phosphorylation step, which is probably not influenced by the drug. The second point of action seems to be the phosphorylation steps between cytochrome b and cytochrome oxidase, which is suggested from the uncoupling effect on succinate-linked phosphorylation.

Kaul and Lewis (61) studied the effects of chlordiazepoxide and other minor tranquillizers on the adenine nucleotides of rat brain. They found that chlordiazepoxide caused a highly significant fall in ATP and a rise in ADP levels. Of the drugs tested, only

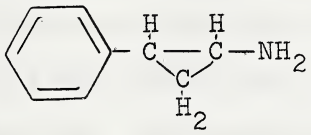
chlordiazepoxide showed a marked behavioral depressant activity which was complicated by the simultaneous occurrence of muscular paralysis. The dosage used was in the same order as those shown by Randall, Schallek, Heise, Keith and Bagdon (55) to cause depression of spontaneous motor activity. It was noted that dosages of meprobamate, which also produced paralysis, had no effect on the adenine nucleotides, although the meprobamate and chlordiazepoxide-treated animals appeared to be equally paralyzed. This difference, they felt, could be explained on the basis of meprobamate acting only at the spinal level, while chlordiazepoxide acts on the brain.

The observations reported by Kaul and Lewis point to a reasonably consistent association between behavioral changes among tranquillizers and anti-depressants and alterations in brain adenine nucleotide levels. These effects are more consistent than other properties possessed by these compounds, for example, their effects on brain amine levels. It is possible that depressants may reduce the amount of energy available by interfering with ATP synthesis or by depressing the efficiency with which energy is utilized. The work by Kaul and Lewis agrees with other observations reported which would indicate that a decrease of ATP is expected, since chlordiazepoxide appears to interfere with the metabolism of glucose and oxidative phosphorylation of rat brain mitochondria.

TRANYLCYPROMINE

I PHARMACOLOGY

Tranylcypromine was synthesized by Berger and Yost, with the intention of incorporating into the same molecule the anesthetic cyclopropane and the central stimulant amphetamine (62). Tranylcypromine is trans-2-phenylcyclopropylamine, and may be structurally represented as:



This compound is classified as a nonhydrazine monoamine oxidase inhibitor (MAO inhibitor) as well as an antidepressant. Nonhydrazine types of MAO inhibitors resemble the hydrazines in their ability to cause an increase in the concentrations of brain monoamines, such as serotonin and norepinephrine, and to inhibit the depletion of these amines by reserpine and related drugs. Although the ability to increase amine levels seems to be a common property of *in vivo* inhibitors of MAO, a causal relationship between this action and MAO inhibition has not been well established. Accumulated evidence necessitates the conclusion that many of the effects of the MAO inhibitors do not result in an inhibition of MAO *per se*, but relate to the binding of amines in the tissues or to a direct pharmacological action of the drug itself (63).

Tranylcypromine is a potent MAO inhibitor both *in vivo* and *in vitro*. The *in vitro* preparations are unusual because preincubation in the absence of oxygen results in a greater degree of inhibition (64). The duration of *in vivo* inhibition produced

by tranylcypromine is consistent with the fact that the effects of this inhibitor are not readily reversible *in vitro*. The drug has a relatively rapid onset of action, and the effects may last for one or more days (64).

Tranylcypromine closely resembles amphetamine, exhibiting signs of central stimulatory and sympathomimetic activity immediately after injection, as well as producing an immediate rise in blood pressure (64). The amphetamine-like anti-anxiety action occurs before any inhibition of MAO is observed (65) and is unrelated to its MAO-inhibiting properties (66). Metabolism studies have shown that the breakdown of tranylcypromine does not involve the formation of amphetamine (62).

Although tranylcypromine elicits an immediate pressor response, this effect is later reversed and hypotension develops, an adverse effect associated with the drug. Selective blocking of the pressor effect indicates that it is peripheral in origin (64).

II EFFECT ON ADENINE NUCLEOTIDES

The effects of tranylcypromine on other enzyme systems has not been studied extensively. Some studies have been carried out on diamine oxidase, and aerobic dehydrogenase associated with flavin adenine dinucleotide (FAD), and the general opinion is that tranylcypromine has little effect on these enzymes and only then at very high concentrations (64). Lewis and Pollock (67) have shown that tranylcypromine did not affect rat brain ATPase.

Gey and Pletcher (68) found that tranylcypromine produced increased levels of lactic and pyruvic acids in rat blood. The acid levels reached their maxima in two or three hours and were still in evidence after fifty hours, paralleling MAO inhibition. The authors concluded that MAO inhibitors enhance glycolysis by altering monoamine metabolism which is reflected in increased pyruvic and lactic acid levels.

Lewis and Van Petten (69) studied the effects of antidepressant drugs on the adenine nucleotides of rat brain. They found that tranylcypromine produced a significant increase in ATP levels and a decrease in ADP levels, significantly increasing the ATP/ADP ratio. However, by increasing the dosage and studying various time intervals they found that this ratio returned to control levels somewhere between 6 and 12 hours after administration. There also appeared to be a relationship between the central stimulant action observed and an increase in the ATP/ADP ratio. This could be due to a decreased utilization or an increased resynthesis of ATP. The increase in brain ATP levels during central nervous system stimulation by drugs, presumably when the brain also requires more ATP for its function, indicates a net

increase in resynthesis.

From these results no relationship is indicated between the abilities of a drug to inhibit MAO or to increase brain levels of ATP. Similarly, there appears to be no relationship between the level of brain ATP and increased levels of serotonin or norepinephrine. Nor would the results indicate a simple relationship between the increased levels of ATP and the synthesis, binding, or metabolism of norepinephrine.

The relatively close agreement between the drugs used in this study to increase the brain levels of ATP, and to produce behavioral signs of central stimulation in the rat, perhaps indicates an important function for this labile phosphate in the action mechanism of the antidepressants. It is possible that an increased level of ATP may influence the maintenance of ionic gradients in the neurones of the brain, and may manifest itself as behavioral changes mediated by biogenic amines. Whatever the process, the results indicate a shift in the dynamic relationship between utilization and production of ATP which may be an important factor in the action of antidepressant compounds (69).

ADENOSINE TRIPHOSPHATE

I GENERAL CONSIDERATIONS

ATP is generally accepted as the most important labile carrier of metabolically available energy in cells. It is the end product of three major cellular energy-generating systems; glycolysis, oxidative phosphorylation, and photosynthesis. The basic reaction underlying the formation of metabolically utilizable energy is the transfer of a pair of electrons from a donor to an acceptor. The primary hydrogen donors are derived from that portion of nutrients which can be subjected to intramolecular modulations of chemical structure, permitting the sequential withdrawal of electron pairs. These electron pairs are transported by means of the respiratory enzymes to oxygen, the final electron sink of mitochondrial metabolism. The respiratory enzymes are proteins which contain three coenzymes or prosthetic groups, which function as electron carriers: pyridine nucleotides, flavin nucleotides, and iron porphyrins (32).

If the purpose of carbohydrate and lipid metabolism is the generation of high energy phosphate compounds to be utilized for endergonic purposes, it is apparent that this is achieved primarily through the oxidation of the reduced coenzymes, and not from the immediate oxidation of the various metabolites elaborated from glucose or lipid. However, little is known of the mechanism whereby electron transport is coupled with the formation of ATP. Of the three moles of ATP generated incident to electron transport from DPNH to oxygen, it is thought that one arises during transport from DPNH to the flavoprotein, a second is associated with the oxidation of cytochrome b by cytochrome c,

and the third with the oxidation of cytochrome c by cytochrome oxidase (32, 70). It is agreed that associated with these discrete oxidative steps there is formation of an energized intermediate which can ultimately join inorganic phosphate with ADP to form ATP (71).

The utilization of ATP to drive the diverse energy requiring processes of the cell, such as active transport of ions (72) and muscle contraction (73), increases the supplies of ADP and inorganic phosphate, which in turn become available to react with the coupling mechanism and the continuation of respiration.

II SEPARATION

The trichloracetic or perchloric acid extracts of tissue yield the acid-soluble phosphates, such as adenosine polyphosphates, phosphocreatine, and inorganic phosphorus (74). To obtain reliable estimates of the quantities of these extremely labile compounds, the tissue should be frozen in situ, preferably with liquid air, liquid oxygen, or liquid nitrogen, and removed while still frozen. The frozen tissue can then be ground to a fine powder in a prechilled grinder and added to the acid denaturant, or can be ground directly in the acid.

Weiner (75), studying the critical factors influencing the assay of rat brain adenine nucleotides and phosphocreatine, found that decapitation before immersion of the severed head into liquid oxygen results in lower levels of ATP and phosphocreatine, and higher levels of ADP and AMP, than if the animal is plunged directly into the liquid coolant. He also found that the activities of ATPase and creatine phosphokinase of the brain are sufficiently great to enable the destruction of all ATP and phosphocreatine to take place within a few seconds. In this regard he demonstrated that ATP is hydrolysed fairly rapidly at temperatures as low as 4° C. From his results and the discussion of Heald (74), it appears that the maintenance of low temperatures for the extraction of ATP is imperative.

The fractionation of the acid-extracted phosphates can then be performed as outlined by Heald (74). The adenine nucleotides and inorganic phosphorus are precipitated as calcium salts. An alternative fractionation procedure is based on the different solubilities of the barium salts of the phosphates. The nucleotides

may also be quantitatively precipitated as the mercury salts. However, these methods are not suitable for work with radioactive phosphorus, and require supplemental chromatography to avoid errors introduced by overlapping of the various fractions and the consequent contamination with radiophosphorus.

The application of various chromatographic techniques to this problem has made the separation of the nucleotides from the acid-soluble fraction possible. These techniques include paper chromatography, column chromatography, electrophoretic chromatography, and thin-layer chromatography.

The use of paper chromatography for the separation of the acid-soluble phosphates was first discussed by Hanes and Isherwood (76). Since then, many reports have appeared describing improvements in the chromatographic techniques. Diamond (38) used a two-solvent, two-dimensional system for the separation of the acid-soluble nucleotides and inorganic phosphorus. In his work he has discussed the attributes and drawbacks of many systems. However, the main disadvantage to using paper chromatography is the long development time.

The detailed procedure for the separation of the nucleotides and nucleosides and other derivatives by ion-exchange columns was discussed by Hurlbert, Schmitz, Brumm, and Potter (77) with regard to a Dowex formate resin. There is agreement, however, that when the nucleotides are exposed to low pH values for a long period of time the acid-labile compounds are broken down (78,79). In an attempt to combat this breakdown, Imai and Berne (78) used a Dowex bicarbonate resin and obtained excellent results. Ion-exchange chromatography, while based on a simple principle and

effecting excellent resolution of the components, is time-consuming, requires large amounts of sample, and allows only one sample to be chromatographed at a time. It does offer one distinct advantage and that is the automation of the operation once the column is established.

High-voltage paper electrophoresis has been employed for the separation of the nucleotides from the acid-soluble extract (79) and for the separation of the adenine nucleotides (80). The separation of the components was excellent and the time required for the separation was short. Both groups employed buffer systems of pH less than 4.0, carried out the separation at reduced temperatures, and used acid pre-treated paper strips. Sato, Thomson, and Danforth (80) found that zinc ions were necessary as carriers to stabilize the labile phosphates. The techniques involved are somewhat complex and require specialized equipment.

Randerath (81) utilized ion-exchange resins of cellulose, as well as unmodified cellulose , to separate the various nucleotides by thin-layer chromatography (TLC). He found that TLC is appreciably more sensitive, the quantity that can be chromatographed without tailing is greater, and the time for development much shorter than paper chromatography. With the ion-exchange cellulose resins the development time is extremely short, usually less than 45 minutes. The publication of his book and its subsequent translation into English (82) provides an excellent reference to TLC of the nucleotides as well as those other areas where chromatographic techniques are applicable. Books by Sthal (83) and Bobbit (84) also contain pertinent information on the TLC of nucleotides.

III ANALYSIS

Assay procedures for ATP depend, in general, on the unique physical, chemical, and enzymatic properties of this compound. Among these properties are the absorption spectrum of the purine component, acid lability of the polyphosphate linkage, and the specificity of ATP in various enzymatic reactions.

Most of the enzymatic determinations of ATP are based on differential spectrophotometry of purine compounds utilizing specific enzymes, a system designed by Kalckar (85). The method relies on the spectrophotometric changes in the products after enzymatic attack. Both Lewis and Van Petten (86) and Weiner (75) have used this technique with minor modifications which they felt were necessary to suit their particular needs. Heald (74) in his discussion of ATP measurement mentions several other modifications.

Strehler and Totter (87) point out the usefulness of the luciferin-luciferase method for ATP determination. ATP can be measured directly by making use of the linear relationship between the amount of light produced and ATP concentration. This method is specific for ATP since other phosphorylated compounds are inactive (88). Now that there are commercial enzyme supplies available, this method would appear to be superior to other methods, provided that only the concentration of ATP is required.

The specific enzyme methods for the estimation of the adenine nucleotides appear to be rapid and accurate, but they are not suitable for radioactive studies since no actual separation of the individual nucleotides is carried out.

Chemical methods are generally based on the amount of inorganic phosphorus released when the polyphosphates are hydrolysed in acid and using high temperatures. The method of Fiske and Subbarow (89), utilizing ammonium molybdate and aminonaphtholsulfonate, has been used extensively for this purpose. A blue color is produced which can be measured either spectrophotometrically or colorimetrically. There have been many variations of this procedure since its appearance in the literature, each modification claiming an advantage over its predecessor. Basically they are all the same with approximately the same sensitivity, and they differ only in reducing agents, the use or absence of heat, and the stability of the complex formed (90).

The estimation of the adenine nucleotides can also be accomplished by determining their ultraviolet absorbance at 260 millimicrons. As in the case of the phosphorus determination, the nucleotides must be separated from one another to provide an accurate estimation. The separation of the phosphate group in a nucleotide, from the effective ultraviolet chromaphore by the saturated carbon chain of the sugar residue, may be expected to produce very little changes in the absorption characteristics. This is in fact the case, and many nucleotides may be regarded as barely distinguishable from their parent nucleosides in the present state of ultraviolet technique (91).

EXPERIMENTAL

ANIMALS

Male rats of the Sprague-Dawley strain, weighing between 110 and 145 grams, were used in this experiment. They were housed eight to ten animals per cage and were maintained in a standard animal room. The diet consisted of Purina Lab Chow and tap water ad libitum. The animals were weighed frequently to accustom them to being handled and every effort was made to avoid excitement prior to the killing of the animal. Before the experiment was begun, two animals were removed from their cages in advance of the regular feeding time, weighed, and placed in a cage located in the research area.

INJECTIONS

All drug injections were given intraperitoneally using light ether anesthetic. The following drugs were used in the form of their commercially available parenteral solutions:

chlorpromazine (Largactil - Poulenc Limited), 25 mg/ml;
reserpine (Serpasil - Ciba Company Limited), 2.5 mg/ml;
chlordiazepoxide (Librium - Hoffman-La Roche Limited) 50 mg/ml.

Tranylcypromine (Parnate - Smith Kline and French Limited) was obtained from the company in its powdered form, and was dissolved in 0.9% saline to give a solution of 10 mg/ml. The control animals were given a solution of 0.9% saline. In all cases the animals received 1 ml/kg of body weight, with no injection exceeding 0.15 ml. Injections were made five hours before killing.

A typical lethargic condition was observed in those animals treated with chlorpromazine, reserpine and chlordiazepoxide, with

the chlorpromazine and chlordiazepoxide-treated animals showing a more pronounced state of sedation than the reserpinized animals. Those animals treated with tranylcypromine showed no signs of sedation and were inclined to be hyperexcited. At no time did any of the animals lose consciousness, and those exhibiting signs of sedation could be aroused from their lethargy by gentle stimulation.

One hour after the administration of the drug, the animals were injected intraperitoneally with 200 microcuries of P^{32} solution regardless of the individual body weight. It has been shown that doses much larger than 200 microcuries are necessary to produce metabolic changes due to radiation (92,93).

TISSUES

The animals were killed by plunging them headfirst into liquid air contained in a wide-mouth Dewar flask. The whole body was immersed in the liquid air for about 10 seconds, and then withdrawn until just the head remained. The head was allowed to remain 10 seconds longer, then was chiseled off and returned to the liquid air. The body was held over a heparinized centrifuge tube and approximately 1 ml of whole blood was collected.

The heart, mid-lobe of liver, and the adrenals were excised as rapidly as possible, blotted, and placed in liquid air until used. The body was normally frozen only to a depth of 0.5 cm although some of the organs, in particular, the liver and the fatty areas around the kidneys, showed signs of being frozen. The whole brain was rapidly chiseled free of the skull and ground to a fine powder in a mortar, pre-chilled with liquid air. Approximately 300 mg of brain tissue were added to a tared

centrifuge tube containing 2.5 ml of 2% perchloric acid chilled in ice. A thin, glass stirring rod was used to break the ice crust formed at the tissue-acid interface to expose the tissue to the protein denaturant as soon as possible. The tared centrifuge tube was reweighed, and the weight of the tissue sample obtained by difference. Liver and heart tissue were treated in a similar manner. The adrenal glands were placed in a tared homogenizing tube containing 1 ml of 2% perchloric acid, reweighed, and homogenized. The tubes were centrifuged for 20 minutes at -4° C at 3000 rpm (1000 g) in a Servall refrigerated centrifuge.

The supernatant was decanted into ice-cooled graduated centrifuge tubes and treated with 1N potassium bicarbonate which precipitated the excess perchloric acid and brought the pH to about 6 as indicated by universal indicator paper. The neutralized material was centrifuged for the same length of time under the same conditions as mentioned previously, in order to precipitate all the potassium perchlorate. The tubes were removed to an ice bath, the volume noted, and retained for chromatography.

The tubes containing whole blood were centrifuged at room temperature for 20 minutes at 2000 rpm in an International Centrifuge, Model CS. An aliquot of 0.1 ml of plasma was pipetted into 15 ml of liquid scintillation counting mixture and this was stored in a closed cupboard until required for counting.

CHROMATOGRAPHY

Thin-layer chromatography was chosen for the separation of the nucleotides as experience proved it to be more reliable and more accurate than with the method of Sato *et al* (80).

The support for the thin-layer chromatograms was

MN-cellulose powder 300 without binder (Macherey, Nagel and Co., Düren, Germany). A slurry was made by adding 10 grams of the powder to 60 ml of demineralized water in a Waring Blendor, Model PB-5A, and homogenizing for 1 minute. This was sufficient material to coat five, 20 X 20 cm, clean, glass plates with a layer 0.25 mm thick. The glass plates were cleaned in chromic acid prior to use since plates with greasy areas will not hold a layer. A Desaga spreader and spreading tray were used to coat the plates in the manner of Sthal (94). The plates were placed in a rack and stored in an electric oven at 55° C.

Before applying the sample to the plate, a heavy line was drawn through the layer 17 cm from the bottom of the plate. This marked the limit to be reached by the developing solvent. The plate was divided into four columns, each 5 cm wide, from side to side providing four individual development areas on one plate. One outside column was used for a standard solution of ATP and the other provided a blank. The two inside columns were used to develop the samples; one for the phosphorus determination, and the other for the radioactive determination. Thus, there was a standard, a blank, and two samples all on one plate.

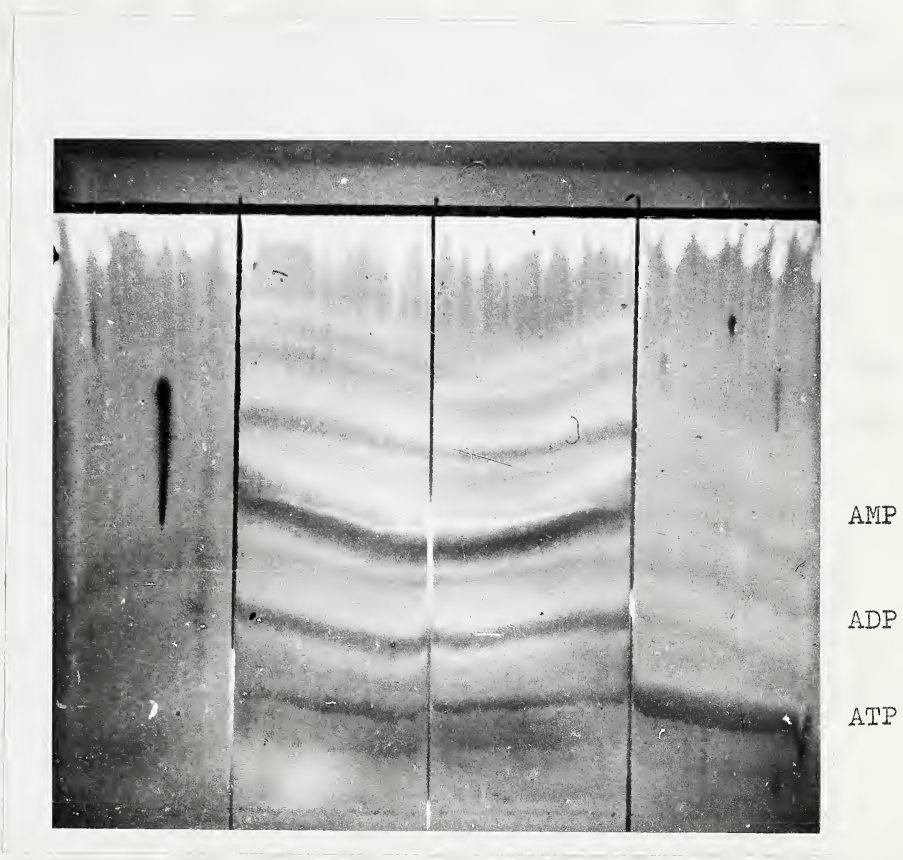
The samples were applied 15 cm from the pre-determined solvent front as narrow bands across the column on the plate. Experience showed that bands no wider than 0.5 cm resulted in a sharper separation without tailing than if the sample were applied as a spot. The samples were applied in 75 microliter aliquots for brain, liver, and heart tissue, and in a 100 microliter aliquot for the adrenal glands.

The plates were placed in covered, glass developing tanks, 24 cm long, 24 cm high, and 12 cm wide, containing 125 ml of solvent. Both the tank and the matching cover had ground glass edges to effect a tight seal. The solvent, from Randerath (95), consisted of tertiary-amyl alcohol, formic acid, and water in the ratio of 3:2:1 by volume. This was added to the tanks sufficiently in advance of chromatography to allow for saturation and equilibration of the atmosphere within the tank. It was found that if the tanks were placed in a cupboard they developed faster and the solvent front migrated more uniformly than if the tanks were allowed to stand on the bench top.

After development, which required about 3 hours, the plates were taken out and allowed to dry at room temperature. They were then viewed under a short-wave ultra-violet lamp, at 2537 Å, using a Mineralight (Ultra-violet Products, San Gabriel, California). The nucleotides appeared as dark -blue bands on a light blue background. Visualization of the nucleotides was more pronounced if the ultra-violet light was allowed to shine through the layer and then the glass of an upright plate, than if the light was shone on the cellulose layer of a plate which was laying flat. In all cases the ATP standard solution was used as a reference marker for locating the ATP band in the sample. The area delineating ATP was marked off with a stylus.

Preliminary studies with standard solutions of the adenine nucleotides and tissue samples showed that the Rf values were similar to those listed by Randerath (95). The values for ATP were from 0.16 to 0.20, for ADP were from 0.30 to 0.35 and

Figure 1



Ultraviolet Photograph
of a Developed Thin-layer Plate

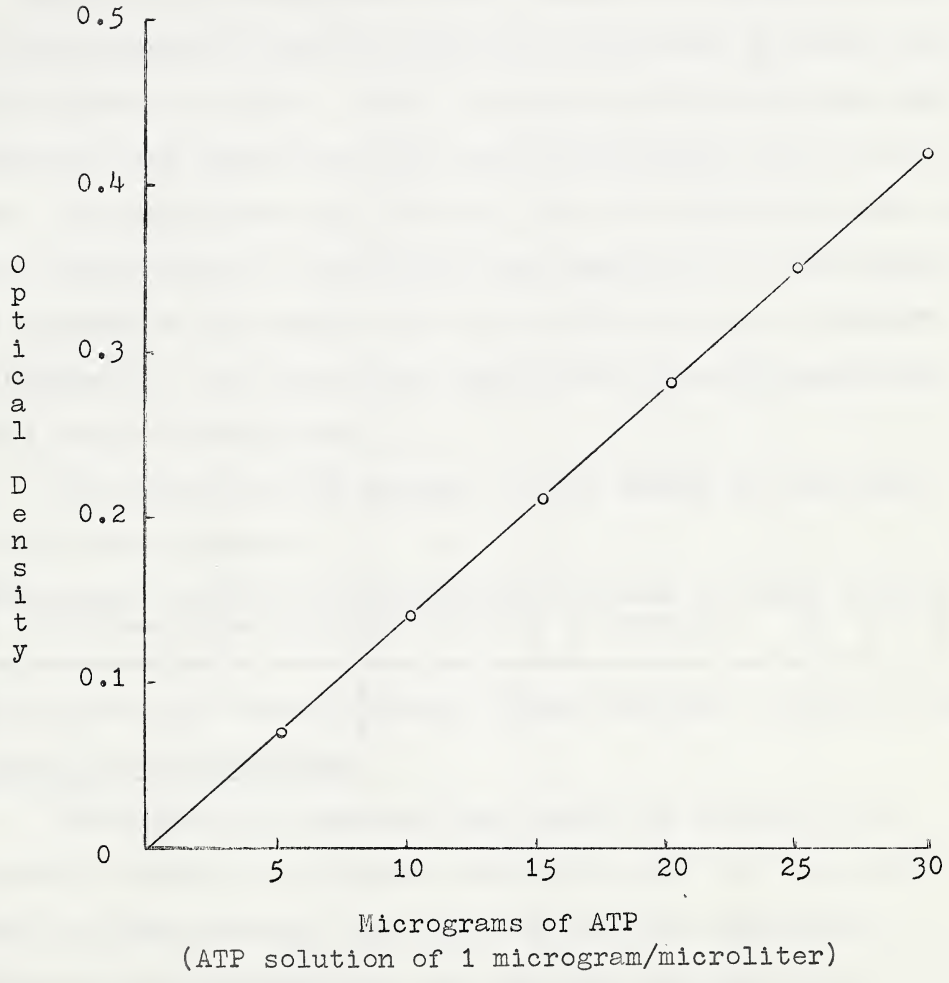
for AMP were from 0.50 to 0.60. Using an Actigraph radiation scanner (Nuclear-Chicago Corporation), modified for thin-layer plates, and a spray for locating inorganic phosphorus (96), it was found that the inorganic phosphorus was closely associated with AMP. Scanning of the plate with the Actigraph showed that most of the radioactive material on the plate was due to the inorganic phosphorus and AMP. Both ADP and ATP showed activity above background levels, with the former being somewhat more active. The areas of activity were well defined and there was little, if any, overlapping.

ESTIMATION OF ATP

Preliminary work on the estimation of ATP using both the ultra-violet absorbance and phosphorus content were carried out. Both proved to be satisfactory, but the ultra-violet technique was not quite as sensitive as the phosphorus determination due to the small size of the sample and the dilution necessary in eluting it. However, it did serve to verify the presence of the adenine moiety in the sample. The use of the phosphorus method for the estimation of ATP was convenient for the calculations of specific activity.

The method of Lucenda-Conde and Prat (97) as modified by Diamond (38) was used to measure the phosphorus present. The ATP standard, one sample and an appropriate area of the blank, were scraped off the plate by means of a scalpel directly into a Kjeldahl flask of 10 ml capacity. To this was added 0.7 ml of a mixture of concentrated sulfuric acid and 60% perchloric acid in the ratio of 3:2 by volume. This solution was wet-ashed on an electric Kjeldahl heater until the solution remained colorless.

Figure 2



The Relationship Between Optical Density and ATP Concentration at 785 Millimicrons

The ashed material was allowed to cool, then 2.0 ml of demineralized water was added. After this had cooled, two drops of bromothymol blue indicator were added, and the solution was titrated with concentrated ammonium hydroxide until one drop turned the solution blue. One ml of acid-molybdate color reagent was added and the flasks were placed in boiling water for 30 minutes to allow for the development of color. After cooling, the solutions were read in a Beckman DK-2 ratio-recording spectrophotometer at 785 millimicrons. The instrument was balanced with the blank solutions and reading obtained for the phosphorus was indicative of the amount of ATP present in the sample which was obtained from a standard curve (Figure 2). It can be seen that Beer's Law was satisfied over the range investigated.

The amount of ATP present in the sample was obtained in the following manner:

$$\frac{\left[\begin{array}{c}\text{micrograms of ATP in chromatogram} \\ \text{(obtained from standard curve)}\end{array}\right] \times \left[\begin{array}{c}\text{volume of acid} \\ \text{extract}\end{array}\right] \times \left[1000\right]}{\left[\text{milligrams of tissue}\right] \times \left[\text{volume chromatographed in microliters}\right]}$$

RADIOACTIVITY DETERMINATION

Scintillation counting was chosen for radioactivity measurements because of its high sensitivity and the low counts observed in brain tissue. The solution from the phosphorus determination was incompatible with any counting mixture so that it could not be used for radioactive determinations.

The duplicated tissue sample was scraped off the plate into a centrifuge tube. This was also done with a portion of the blank column. One ml of demineralized water was added and the cellulose was allowed to hydrate. After hydration, usually

about 5 minutes, the mixture was stirred with a thin, glass stirring rod to break up any large pieces of cellulose and to aid elution. The suspension was centrifuged at room temperature for 20 minutes at 2000 rpm. The supernatant was decanted off into a liquid scintillation vial containing 15 ml of Polyether 611 counting mixture. The vials were capped, swirled, and allowed to dark-adapt in the scintillation counter before being counted. The instrument was set up to count using the channel ratio method to estimate quenching.

A Nuclear-Chicago Liquid Scintillation instrument, Model 723, with two channels was used for the radioactive determinations. The instrument settings were as follows:

Data High Voltage	800
Gate High Voltage	900
Scaler 1 set to Channel 3	
Scaler 2 set to Channel 2	
Channel 3 monitoring L3-L4	
Channel 2 monitoring L3-L5	
Level 3 setting 0.5 volts	
Level 4 setting 2.5 volts	
Level 5 setting 9.9 volts	

A C^{14} unquenched sample was used as reference standard to check the instrument settings, as P^{32} standards decay too soon to be of value as reference standards. When a ratio of $0.9985 \pm .0001$ was established with the C^{14} standard the instrument was ready for counting.

The samples were counted for 10 minutes. The counts for Scaler 2 were taken, the background as obtained from the

blank was subtracted, and the counts were corrected for decay. The corrected counts and corresponding channel ratios were taken to a previously prepared quenching curve (Figure 3) and the disintegrations per minute were calculated.

The quenching curve was prepared by taking aliquots of standardized radiophosphorus solutions and counting them so that the ratio of Scaler 1 to Scaler 2 was about 0.30 (98). Water was added to the samples which had the effect of shifting the energy peak to a lower value. The samples were recounted. Since the theoretical disintegrations per minute are known and the instrument provides the counts per minute as well as the channel ratio, percent efficiency can be plotted against channel ratio. Subsequent additions of quenching agent will change the ratio to higher values as the energy peak is shifted out of the counting window, until both Scaler 1 and Scaler 2 values are the same. The portion of the graph between 0.4 and 0.8 channel ratios should be a straight line, and the absolute counting rate can be determined from this.

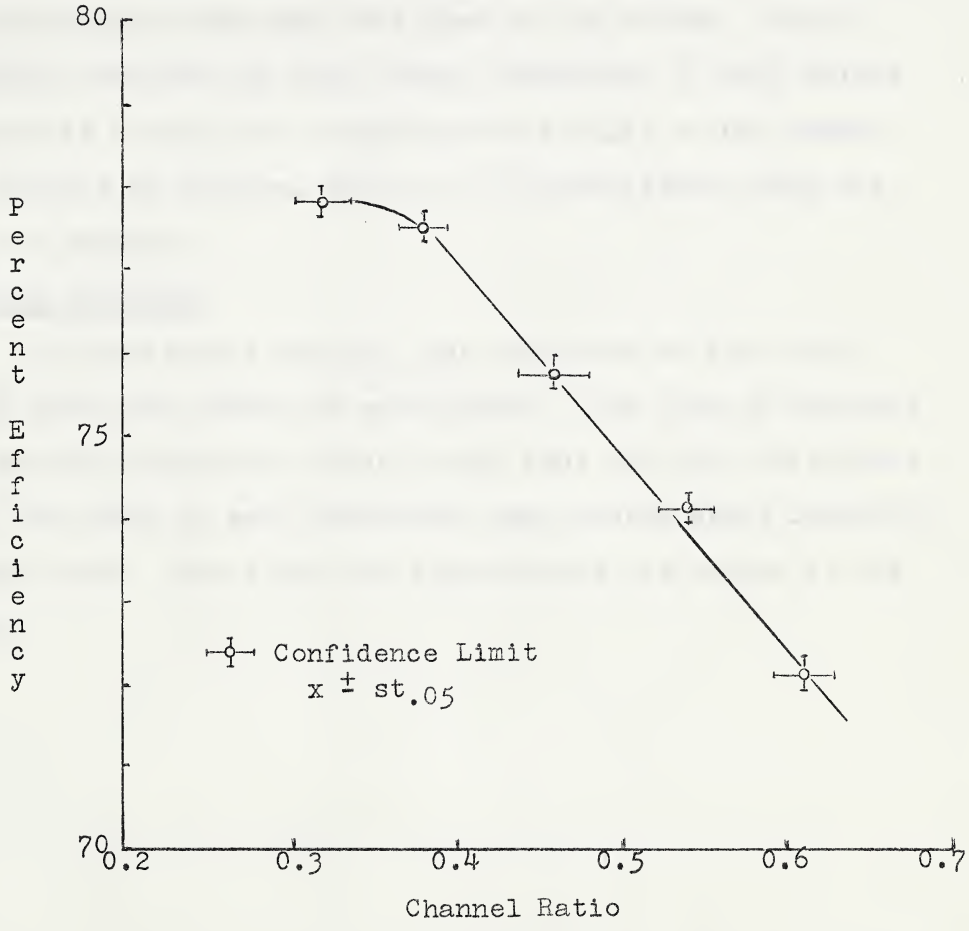
DEFINITION OF TERMS

The specific activity of a sample (SA) refers to the disintegrations per minute divided by the number of micrograms of phosphorus present in the sample.

$$SA = \frac{\text{disintegrations per minute}}{\text{micrograms of phosphorus}}$$

The plasma specific activity (PSA) refers to the disintegrations per minute of plasma divided by the volume of plasma used. The plasma corrected specific activity (PCSA) is the PSA multiplied by the animal body weight, i.e. corrected for body weight.

Figure 3



Quenching Curve for P^{32} in Polyether 611

The relative specific activity (RSA) refers to the SA of the sample divided by the PSA. This relates the SA of the sample to that of the plasma and takes into account the dilution of the injected radio-phosphorus as well as any small differences there may have been in the dosage. Since all animals received the same dosage regardless of body weight there must be a basis for comparing the results of one animal to the results of another, and this is accomplished using the RSA of the animals.

STATISTICAL ANALYSIS

A statistical analysis was performed on the means obtained from each series of experiments. The type of analysis chosen was the randomized block design (99) and the differences between the means of each experiment were tested using Dunnett's procedure (100). The level for significance was chosen at the 5% level.

RESULTS

The results from the experiments performed are tabulated as follows: Tables I to IV are concerned with ATP concentration, the specific activity and the relative specific activity of the various tissues studied, while Table V lists the plasma specific activities corrected for body weight.

It can be seen from Table I that there is no significant alteration in the concentration of ATP in brain tissue in any of the groups of experiments done. The specific activities in Table I show that chlorpromazine did significantly increase the specific activity in brain tissue, but when calculated relative to the specific activity of the plasma, a decrease is observed. None of the other drugs had any effect on the incorporation of P^{32} into the ATP.

Table II presents a comparison of the effect of the same features of ATP in liver tissue. There was no significant change in ATP concentration in those animals treated with chlorpromazine, while each of the other drugs induced a significant decrease. All four drugs significantly decreased the specific activity of liver, but chlorpromazine was the only drug to alter the relative specific activity, where a significant decrease is also apparent.

In Table III the results of the drug effects on heart tissue are shown. Those animals treated with chlorpromazine show no significant change while the values for the other drugs are significantly lower than control values. The specific activities show a marked decrease with the exception of the reserpinized animals. The relative specific activities are

significantly changed with chlorpromazine and chlordiazepoxide but not with reserpine or tranylcypromine.

Table IV lists the results obtained from the adrenal glands. Again those animals treated with chlorpromazine have no significant change in ATP concentration while the other drugs have shown a decrease. Both chlorpromazine and chlordiazepoxide have reduced the specific activity values but only chlorpromazine resulted in a markedly reduced relative specific activity.

Table V shows the plasma specific activities corrected for body weight. The animals treated with chlorpromazine are the only ones to show a significant change, an increase.

In summary, chlorpromazine has not produced any significant change in the ATP concentration of the tissues studied, but in all cases except for the adrenal gland, the values obtained were somewhat higher than the control values. Chlorpromazine also produced a marked decrease in the relative specific activity of all tissues, as well as the specific activity of liver, heart, and adrenal gland. The brain specific activity was increased, as was that of the plasma, by chlorpromazine.

Reserpine has shown a tendency to lower the concentration of ATP in all tissues except brain. However, the specific activity of the ATP of liver tissue was the only one to be significantly reduced.

Chlordiazepoxide has also shown a tendency to lower all values. It has significantly reduced the ATP concentration and specific activities of all tissues except brain.

Tranylcypromine, the only anti-depressant studied, has no significant effects on the brain tissue, although both ATP concentration and specific activity are above control levels. The concentration values and specific activities of the other tissues are significantly reduced, with the exception of the specific activity of the adrenal glands which is reduced, but not significantly.

Table I

ATP Concentration and Uptake of P^{32} in Brain Tissue

Treatment	Concentration micromoles per gram of frozen tissue	SA	RSA x 10^7
Control	2.55 ± .15	46.5 ± 5.8	81.6 ± 15.5
Chlorpromazine 25 mg/kg	2.67 ± .17	62.6 ± 17.4**	57.3 ± 18.4
Reserpine 2.5 mg/kg	2.57 ± .27	48.5 ± 10.2	81.2 ± 25.2
Chlordiazepoxide 50 mg/kg	2.33 ± .17	44.6 ± 7.1	88.7 ± 8.4
Tranylcypromine 10 mg/kg	2.66 ± .14	51.4 ± 7.4	88.8 ± 13.0

Each value represents the mean of 10 animals ± standard deviation

* Significantly different from control at 5% level.

** Significantly different from control at 1% level.

Table II

ATP Concentration and Uptake of P^{32} in Liver Tissue

Treatment	Concentration micromoles per gram of frozen tissue	SA	RSA x 10^7
Control	2.54 ± .20	905.5 ± 15.9	138.4 ± 11.5
Chlorpromazine 25 mg/kg	2.66 ± .21	758.2 ± 28.4**	76.3 ± 15.6**
Reserpine 2.5 mg/kg	1.95 ± .43**	717.4 ± 35.0**	120.4 ± 28.1
Chlordiazepoxide 50 mg/kg	1.70 ± .22**	647.3 ± 24.3**	118.0 ± 12.7
Tranylcypromine 10 mg/kg	2.08 ± .19**	649.2 ± 36.9**	119.5 ± 13.3

Each value represents the mean of 10 animals ± standard deviation.

* Significantly different from control at 5% level.

** Significantly different from control at 1% level.

Table III

ATP Concentration and Uptake of P^{32} in Heart Tissue

Treatment	Concentration micromoles per gram of frozen tissue	SA	RSA x 10^7
Control	2.75 ± .31	732.0 ± 31.6	116.1 ± 17.3
Chlorpromazine 25 mg/kg	3.04 ± .24	571.7 ± 56.4*	60.6 ± 19.6**
Reserpine 2.5 mg/kg	2.36 ± .42*	642.4 ± 43.3	107.9 ± 26.7
Chlordiazepoxide 50 mg/kg	2.41 ± .15*	531.6 ± 36.0**	96.2 ± 14.5*
Tranylcypromine 10 mg/kg	2.38 ± .20*	578.3 ± 24.5*	103.4 ± 12.7

Each value represents the mean of 10 animals ± standard deviation.

* Significantly different from control at 5% level.

** Significantly different from control at 1% level.

Table IV

ATP Concentration and Uptake of P^{32} in Adrenal Glands

Treatment	Concentration micromoles per gram of frozen tissue	SA	RSA x 10^7
Control	6.31 ± .43	568.0 ± 60.2	83.5 ± 13.2
Chlorpromazine 25 mg/kg	6.09 ± .38	349.5 ± 38.9**	38.0 ± 7.1**
Reserpine 2.5 mg/kg	4.16 ± .42**	505.1 ± 84.5	83.5 ± 20.4
Chlordiazepoxide 50 mg/kg	4.70 ± .61**	409.4 ± 63.1**	77.4 ± 6.5
Tranylcypromine 10 mg/kg	3.17 ± .34**	530.7 ± 34.0	91.5 ± 13.3

Each value represents the mean of 10 animals ± standard deviation.

* Significantly different from control at 5% level.

** Significantly different from control at 1% level.

Table V

Plasma Specific Activities

Corrected for Body Weight

Treatment	Value x 10^{-7}
Control	81.4 ± 8.9
Chlorpromazine 25 mg/kg	131.3 ± 23.0**
Reserpine 2.5 mg/kg	77.5 ± 14.0
Chlordiazepoxide 50 mg/kg	66.5 ± 8.0
Tranylcypromine 10 mg/kg	75.1 ± 5.2

Each value represents the mean of 10 animals

± standard deviation.

* Significantly different from control at 5% level.

** Significantly different from control at 1% level.

DISCUSSION

Before entering into a discussion of the results, some thought must be given to those factors which may contribute to the observed differences. The P^{32}, as inorganic phosphorus, must be absorbed by the blood from the peritoneal cavity. It must then pass from the plasma to the extracellular fluid of the tissue, and in the case of the brain, must also cross the blood-brain barrier. The phosphorus has to cross the cell membrane from the extracellular fluid to the intracellular fluid where it is available to the cellular functions. In order to be utilized in the formation of ATP, inorganic phosphorus must be taken up by the mitochondria. Any alteration in the passage of P^{32} from the plasma to the mitochondria should manifest itself in a subsequent alteration in ATP specific activity (SA), relative specific activity (RSA), or plasma corrected specific activity (PCSA). Any change in synthesis, utilization, or turnover (i.e. rate of formation and breakdown) of ATP should be reflected by changes in both the concentration and specific activity, provided there is no alteration in the incorporation of P^{32} or any change in the cellular inorganic phosphorus pool.

If the PCSA is altered, there would be reason to suspect that the passage of P^{32} from the plasma to the extracellular fluid is impaired or enhanced, depending on the observed change. If there is no change in the PCSA, then a fall in the specific activity relative to the plasma must indicate a reduction of the SA. This could be due to enzymic inhibition of oxidative phosphorylation, inhibition of the uptake of P^{32} by the mitochondria, or even a restriction of

P^{32} to the intracellular fluid by, perhaps, inhibition of the transfer through the cell membrane.

The SA (counts per minute/microgram of ATP-phosphorus) should provide a means for the estimation of the synthesis, utilization, or turnover of ATP, as long as the supply of radio-isotope is unhindered. The situation where the concentration of ATP is unchanged but an elevated SA occurs suggests a facilitated incorporation of the radio-isotope. This may imply that the synthesis rate is increasing; but if the concentration is not increased then the utilization rate must be increased. On the other hand, utilization may be reduced, allowing a slow accumulation of the tagged compound. An unchanged SA could demonstrate the turnover is unchanged, or that synthesis and utilization have been altered to the same extent and in the same direction. A lowered SA suggests a reduced incorporation of P^{32} and presumably, a concomitant decrease in synthesis.

The case where the ATP concentration has been reduced and the SA unchanged suggests that utilization is proceeding at a greater rate than synthesis. If the SA has remained unchanged then the rate of utilization may be increased over the rate of synthesis, but the time chosen for the experiment has not been such as to show the effect. However, synthesis may be reduced, but the pool of labelled inorganic phosphorus may have been greatly increased, such that incorporation of P^{32} is facilitated. A decreased SA implies a reduced synthesis.

The experimental work shows that chlorpromazine significantly increases the PCSA, suggesting that P^{32} is not

being taken up by the tissues. Further emphasis is added to this suggestion as all the RSA values and SA values of liver, heart, and the adrenal glands are significantly reduced. As chlorpromazine is known to affect membrane permeability (11,19), it is possible that the transport of phosphorus across the cell membrane has been impaired. Then the reduced SA values observed in the animals treated with chlorpromazine may be explained as a reduced transfer of P^{32} into the tissues as a result of an altered membrane permeability to phosphorus. This does not eliminate the possibility of a reduced turnover of ATP, as a result of a decreased utilization, which is suggested by Weiner and Huls (36). In all probability it is a combination of a reduced membrane permeability to phosphorus and a decreased turnover of ATP, but the data presented here are not sufficient to allow for more than speculation.

Brain tissue, however, shows an increased SA but an unchanged ATP concentration under the influence of chlorpromazine, suggesting an increased turnover or an enhanced radio-isotope incorporation. Quadbeck (101) found that chlorpromazine increased the uptake of P^{32} into rat brains 25% above control values. This would imply that the blood-brain barrier, under the influence of chlorpromazine, permits the passage of phosphorus into the brain more readily. If this is the case, then the incorporation of the isotope would be facilitated due to an increased availability, resulting in an increased SA.

On the other hand, Kaul et al (39) have shown that chlorpromazine increases the concentration of ATP in the brain

6 hours after drug administration. Since the present experiment was carried out 5 hours after drug administration, it is still possible that the rate of synthesis is greater than the rate of utilization, but has not become great enough to be observable. If the availability of P^{32} is enhanced, an increased incorporation of the radio-isotope is possible, resulting in an increase in the SA but a concentration which is not yet significantly increased. As the PCSA is very high, a significantly increased SA in the brain could still result in a low RSA value, when compared to control animals.

Reserpine showed no effect on the brain tissue of rats. This is in direct contrast to the work of Kaul and Lewis (44) who found a significant decrease in brain ATP concentration from 3 to 12 hours after drug administration, but they used twice the dosage level that was used in the present experiment. Since the PCSA and brain RSA were unchanged, it is probable that adequate P^{32} was available but the drug was not interfering with brain oxidative phosphorylation.

The reduced concentration of ATP and unaltered SA observed in heart and adrenal glands may be a result of the rate of utilization exceeding the rate of synthesis; the rate of synthesis may be unchanged from the normal situation, as indicated by an unchanged incorporation of P^{32}. However, synthesis may be reduced with utilization being unchanged. In both tissues the SA is below control values, but not statistically significant at the 5% level, suggesting that synthesis is declining. This would appear to be the case in

liver, where both ATP concentration and SA are reduced.

The results observed with chlordiazepoxide suggest that this drug has no effect on brain ATP levels. Kaul and Lewis (61) found that 3 hours after administration of this drug there was a decrease in ATP levels, but this decrease was not statistically significant from control levels. They found, however, that there was a significant increase in ADP which produced a significant change in the ATP/ADP ratio, the figure they used for their ultimate comparison with control values. The concentration value of brain ATP obtained from the present experiment is in agreement with their finding, suggesting that synthesis may be impaired. However, the SA of brain does not support this proposal.

The effect of chlordiazepoxide on liver and adrenal glands might indicate that ATP synthesis is impaired, as both the SA and ATP concentration are significantly reduced. In heart tissue all values are reduced significantly which may be due to a decreased synthesis of ATP accompanied by a lessened incorporation of radio-isotope.

Tranylcypromine showed an increased ATP concentration and SA in brain tissue, but this was not borne out statistically. These results are in keeping with Lewis and Van Petten (69) who found that tranylcypromine, 6 hours after administration, significantly increased brain ATP levels. If the present experiment had been continued longer, perhaps the results of Lewis and Van Petten could have been verified.

In tissues other than brain, the concentration of

ATP was significantly reduced, with liver and heart showing a decrease in SA as well. The adrenal glands, while not showing a statistically significant decrease, did show a decrease in SA. This would suggest that in all three tissues synthesis is reduced. The availability of the radio-isotope appears to be unaltered as the PCSA and RSA are not changed from control values.

No definite trends can be established from the present investigation on the effect of these psychoactive drugs on the phosphorylative metabolism of the four tissues studied. It would appear that the psychoactive drugs act on specific areas in the brain rather than on the oxidative phosphorylation of the whole brain. The present investigation suggests that the depressant drugs act independently on brain oxidative phosphorylation rather than depressing it as suggested by Kaul and Lewis (61). The data show that chlorpromazine has a tendency to increase ATP synthesis, that reserpine does not appear to affect synthesis, and that chlordiazepoxide may depress ATP synthesis in the rat brain. Tranylcypromine, an anti-depressant, may cause an increase in brain ATP synthesis.

The data also show that chlorpromazine probably interferes with the cellular uptake of P^{32}, possibly by an effect on the cell membrane, and suggests that the drug does not interfere with oxidative phosphorylation in the intact rat. It would appear that reserpine, while not noticeably affecting the brain, interferes with oxidative phosphorylation of the liver, heart, and the adrenal glands. The general trend for chlordiazepoxide is a reduction of ATP synthesis in those tissues

studied. Tranylcypromine appears to increase the ATP synthesis of brain and reduce synthesis in other tissues. Thus it would seem that these psychoactive compounds have no common action on brain oxidative phosphorylation and manifest themselves by acting through other processes.

It is difficult to draw any decisive conclusions from this study as additional information is needed to augment the data. If the effect of these drugs on the concentration and SA of ADP, as well as the amount and SA of the inorganic phosphorus in the tissues were known, more definite conclusions regarding the availability and turnover of the radio-isotope might be drawn. Moreover, time studies, if conducted on each of the drugs, might confirm some of the trends which were indicated.

CONCLUSIONS

1. Chlorpromazine did not alter the concentration of ATP in any of the tissues studied. It decreased the specific activity of liver and heart tissue and of the adrenal glands, and increased the specific activity in brain tissue. The elevated plasma specific activity would indicate that the transfer of P^{32} from the plasma to the intracellular fluid is impaired, which may be due to an alteration in membrane permeability.
2. Reserpine, chlordiazepoxide, and tranylcypromine did not significantly alter the concentration of ATP or the specific activity of brain tissue. They did lower the ATP concentration and specific activity of the other tissues, suggesting a decrease in ATP synthesis.
3. The psychotherapeutic drugs studied did not have a common action on brain oxidative phosphorylation. This would suggest that they act independently through other mechanisms. With the exception of chlorpromazine, the other drugs appeared to interfere with oxidative phosphorylation in liver, heart, and adrenal tissue.
4. A thin-layer chromatographic technique, using unmodified cellulose as the adsorbent, was found to serve as an effective method for the rapid separation of ATP from the other nucleotides.
5. The estimation of ATP by the determination of the phosphorus according to the method described was found to be very sensitive and efficient.

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APPENDIX

Table I

ATP Concentration, Specific Activity and Relative Specific Activity in Brain, Liver, Heart and Adrenal Glands with No Drug

ATP Concentration
(micromoles/gram of frozen tissue)

	Brain	Liver	Heart	Adrenal
	2.56	2.76	3.27	5.75
	2.36	2.63	2.65	5.88
	2.41	2.69	2.64	7.15
	2.65	2.22	3.29	6.64
	2.81	2.51	2.62	6.64
	2.70	2.53	2.84	6.23
	2.58	2.51	2.60	5.80
	2.64	2.37	2.58	6.26
	2.37	2.32	2.68	6.36
	2.44	2.83	2.31	6.37
Mean	2.55	2.54	2.75	6.31
(± SD)	(± .15)	(± .20)	(± .31)	(± .43)

Specific Activity

	51.3	845.2	750.9	537.4
	43.4	971.8	912.2	579.0
	55.4	896.6	663.8	565.8
	44.7	960.4	759.0	587.8
	46.4	916.1	691.6	519.1
	55.0	845.6	611.1	467.2
	40.5	981.5	892.2	664.0
	47.5	872.1	708.1	660.0
	39.4	879.6	670.5	539.8
	41.2	886.3	661.0	559.7
Mean	46.5	905.5	732.0	568.0
(± SD)	(± 5.8)	(± 15.9)	(± 31.6)	(± 60.2)

Relative Specific Activity x 10^7

	88.2	145.3	129.1	92.4
	72.7	162.7	152.8	97.4
	92.5	149.7	110.8	94.4
	75.5	140.5	104.8	68.5
	70.2	131.6	99.3	74.5
	76.2	127.9	92.4	70.6
	65.3	135.9	123.6	92.0
	107.6	134.2	108.0	101.6
	103.5	131.3	116.8	66.4
	64.2	125.0	123.5	77.6
Mean	81.6	138.4	116.1	83.5
(± SD)	(± 15.5)	(± 11.5)	(± 17.3)	(± 13.2)

Table II

ATP Concentration, Specific Activity and Relative Specific Activity in Brain, Liver, Heart and Adrenal Glands with Chlorpromazine

ATP Concentration
(micromoles/gram of frozen tissue)

	Brain	Liver	Heart	Adrenal
	2.77	2.61	3.16	6.50
	2.41	2.69	3.24	6.15
	2.71	2.45	3.36	5.50
	2.51	2.54	3.17	6.35
	2.57	2.93	3.25	5.85
	2.92	2.70	3.05	5.47
	2.78	3.09	2.73	6.43
	2.89	2.60	2.84	5.94
	2.53	2.41	2.66	6.32
	2.59	2.56	2.89	6.34
Mean	2.67	2.66	3.04	6.09
(± SD)	(± .17)	(± .21)	(± .24)	(± .38)

Specific Activity

	91.6	831.6	960.7	314.0
	86.1	873.8	682.3	345.5
	78.0	737.8	598.7	335.8
	41.8	815.2	448.5	331.5
	48.5	643.9	355.4	366.5
	43.9	769.1	535.9	327.1
	47.6	645.8	373.6	363.4
	67.9	870.2	573.6	305.1
	68.3	651.3	498.0	442.9
	52.3	743.1	690.3	363.0
Mean	62.6	758.2	571.7	349.5
(± SD)	(± 17.4)	(± 28.4)	(± 56.4)	(± 38.9)

Relative Specific Activity x 10^7

	81.3	73.8	85.3	43.0
	72.9	47.6	74.2	34.1
	79.7	74.0	81.5	41.6
	43.5	75.4	61.2	35.3
	44.1	93.2	46.7	38.4
	70.0	67.1	52.0	34.5
	32.2	66.3	85.5	31.5
	43.9	72.1	43.6	52.2
	66.3	101.6	37.1	42.4
	39.4	91.7	38.6	27.3
Mean	57.3	76.3	60.6	38.0
(± SD)	(± 18.4)	(± 15.6)	(± 19.6)	(± 7.1)

Table III

ATP Concentration, Specific Activity and Relative Specific Activity in Brain, Liver, Heart and Adrenal Glands with Reserpine

ATP Concentration
(micromoles/gram of frozen tissue)

	Brain	Liver	Heart	Adrenal
	2.91	2.42	2.17	3.76
	3.30	1.68	2.48	3.74
	2.67	2.03	2.47	5.05
	2.88	1.93	2.60	3.96
	2.93	1.90	2.36	4.31
	1.99	1.71	1.79	4.33
	2.10	1.95	2.11	4.31
	2.30	1.94	2.33	3.86
	2.44	2.34	3.30	4.50
	2.20	1.58	1.94	3.79
Mean	2.57	1.95	2.36	4.16
(± SD)	(± .27)	(± .43	(± .42)	(± .42)

Specific Activity

	52.1	882.5	687.6	484.6
	44.3	708.2	481.2	632.0
	52.8	652.8	781.9	664.3
	68.7	823.7	528.6	423.7
	43.2	558.5	634.0	525.7
	53.0	845.4	648.4	454.3
	46.1	745.6	655.1	465.7
	38.0	574.8	499.8	431.4
	54.4	733.5	750.6	534.8
	31.9	648.7	757.2	435.0
Mean	48.5	717.4	642.4	505.1
(± SD)	(± 10.2)	(± 35.0)	(± 34.3)	(± 84.5)

Relative Specific Activity x 10^7

	90.5	153.3	119.5	84.2
	61.1	97.7	66.4	87.2
	109.5	135.4	162.2	137.8
	132.0	158.3	101.6	81.4
	53.8	69.5	78.9	65.4
	82.7	139.8	101.2	69.3
	81.5	131.8	115.8	82.3
	64.4	97.4	92.5	73.1
	83.6	112.7	115.3	82.2
	53.1	108.0	126.0	72.4
Mean	81.2	120.4	107.9	83.5
(± SD)	(± 25.2)	(± 28.0)	(± 26.7)	(± 20.4)

Table IV

ATP Concentration, Specific Activity and Relative Specific Activity in Brain, Liver, Heart and Adrenal Glands with Chlordiazepoxide

ATP Concentration (micromoles/gram of frozen tissue)

	Brain	Liver	Heart	Adrenal
	2.26	1.73	2.46	4.55
	2.57	1.69	2.41	4.50
	2.15	2.14	2.54	3.47
	2.33	1.62	2.23	5.13
	2.00	1.61	2.30	3.89
	2.53	1.79	2.35	5.23
	2.47	1.48	2.22	5.31
	2.34	1.36	2.72	4.73
	2.25	1.61	2.47	4.92
	2.38	1.93	2.35	5.23
Mean	2.33	1.70	2.41	4.70
(± SD)	(± .17)	(± .22)	(± .15)	(± .61)

Specific Activity

	46.4	625.9	549.4	443.8
	50.5	582.5	804.7	392.2
	44.3	495.5	533.1	341.7
	50.3	627.0	550.3	380.8
	45.6	676.1	419.8	430.6
	30.4	764.1	510.7	353.0
	33.8	617.8	459.9	387.6
	52.3	737.5	492.5	565.0
	47.2	671.8	572.7	415.6
	44.8	674.3	412.5	383.6
Mean	44.6	647.3	531.6	409.4
(± SD)	(± 7.1)	(± 24.3)	(± 36.0)	(± 63.1)

Relative Specific Activity x 10^7

	95.1	103.3	90.7	73.3
	98.7	115.8	106.0	78.0
	77.5	108.8	117.0	75.0
	83.3	123.0	107.9	74.7
	88.2	130.8	81.2	83.3
	84.0	106.9	107.5	74.3
	76.6	105.2	78.3	66.0
	100.3	117.4	78.4	90.0
	88.5	126.0	107.4	77.9
	94.8	142.8	87.3	81.2
Mean	88.7	118.0	96.2	77.4
(± SD)	(± 8.4)	(± 12.7)	(± 14.5)	(± 6.5)

Table V

ATP Concentration, Specific Activity and Relative Specific Activity of Brain, Liver, Heart and Adrenal Glands with Tranylcypromine

ATP Concentration
(micromoles/gram of Frozen tissue)

	Brain	Liver	Heart	Adrenal
	2.85	2.36	2.42	3.35
	2.66	2.29	2.19	3.59
	2.41	1.88	2.64	3.27
	2.81	2.24	2.15	3.69
	2.59	1.87	2.57	3.14
	2.79	2.22	2.30	3.21
	2.73	2.03	2.58	2.58
	2.61	2.02	2.28	2.86
	2.60	2.07	2.11	2.83
	2.50	1.80	2.52	3.20
Mean	2.66	2.08	2.38	3.17
(± SD)	(± .14)	(± .19)	(± .20)	(± .34)

Specific Activity

	42.8	550.4	524.0	478.3
	59.5	480.6	722.3	576.3
	43.7	554.0	555.4	467.4
	58.8	545.4	463.3	518.0
	57.2	782.3	672.0	511.3
	55.8	820.3	594.3	543.1
	40.9	781.9	546.0	529.0
	55.1	659.0	538.5	501.2
	44.8	661.8	632.6	566.6
	55.6	656.3	535.0	618.0
Mean	51.4	649.2	578.3	530.7
(± SD)	(± 7.4)	(± 36.9)	(± 24.5)	(± 34.0)

Relative Specific Activity x 10^7

	89.0	107.4	108.9	99.4
	73.3	138.3	95.4	69.3
	100.7	117.9	119.6	95.5
	107.0	112.2	120.1	101.0
	93.8	140.5	115.6	91.3
	90.1	131.4	91.8	80.2
	70.2	122.2	93.6	90.8
	85.0	113.1	83.1	77.3
	74.7	102.1	105.4	94.4
	104.1	109.4	100.2	115.7
Mean	88.8	119.5	103.4	91.5
(± SD)	(± 13.0)	(± 13.3)	(± 12.7)	(± 13.3)

Table VI

Plasma Specific Activity

Corrected for Body Weight

All values are x 10^{-7}

CON	CPZ	RES	CDZ	TCP
84.4	152.1	74.8	76.9	67.8
83.6	105.8	99.3	66.9	75.3
80.9	135.7	55.9	55.1	74.3
81.9	113.5	61.4	60.7	71.4
87.0	116.3	94.8	71.3	76.2
82.7	130.6	78.2	60.8	81.3
93.9	150.9	72.4	72.2	72.4
71.5	101.1	82.0	77.6	82.3
61.8	132.9	87.9	67.2	81.1
86.4	174.0	67.9	56.7	68.4
Mean 81.4 (± SD) (± 8.9)	131.3 (± 23.0)	77.5 (± 14.0)	66.5 (± 8.0)	75.1 (± 5.2)

CON control

CPZ chlorpromazine

RES reserpine

CDZ chlordiazepoxide

TCP tranylcypromine